THE MAYEDA LABORATORY

AUTHOR(S):

Mayeda, Kanae

THE MAYEDA LABORATORY

Head: Prof. Dr. Kanae Mayeda

With the retirement of Dr. K. Maeda in 1941, this laboratory was amalgamated with the Utzino laboratory.

A series of researches of the chemical composition of microorganisms were undertaken by the use of some non-pathogenic bacteria whose large scale laboratory cultivation is feasible without great difficulties.

1) Chemical Composition of a Certain Acid-fast Bacillus of Rat Leprosy Origin, the So-called Leprosy Bacillus. By Syuzo Akasi and Kunio Tanigami: Rep. Inst. Chem. Res., Kyoto Univ., 8, 33 (1933).
2) Studies on the Acetone-soluble Fat Isolated From the Acid-fast Bacillus of Rat Leprosy Origin. By Syuzo Akasi and Kunio Tanigami: Ibid. **10**, 117 (1939).
3) Studies on the Proteins of the Acid-fast Bacillus of Rat Leprosy Origin. By Syuzo Akasi and Fumio Itami: Ibid. **11**, 197 (1941).
4) Chemical Composition of the Subtilis Bacteria. By Syuzo Akasi and Fumio Itami: Ibid. **12**, 195 (1941).
5) Chemical Composition of Some Bacteria. By Syuzo Akasi and Fumio Itami: Ibid. **13**, 1 (1944).
6) Chemical Composition of the Sarcina and the Staphylococcus Bacteria. By Syuzo Akasi: Nippon Seikagaku Kaishi (brief report), **17**, 173 (1943).
7) Studies on "Subtilin", a Wax-like Substance Isolated from Stubtilis Bacteria. By Syuzo Akasi: Ibid. (brief report), **18**, 303 (1944).

Results of Research

Leprosy Bacillus

The amount of 10.1 kgs. of moist bacillus material was analyzed according to the method developed by R. J. Anderson. With regard to the lipid fractions our bacillus was characterized by the predominance of total lipids, especially of the acetone-soluble fat and an exceedingly low content of wax and also with an appreciably high content of polysaccharide in lipids.

Acetone-soluble fat. ... This was merely a kind of glyceride, and polysaccharide, sterol and leprosol were not detected in this fraction. The liquid fatty acids consisted of approximately 2/3 of liquid saturated acids associated with other unidentified acids and of 1/3 of unsaturated acids represented by oleic acid.

Protein. ... This was isolated in several fractions by varying the alkalinity of extracting fluid, and the fractionated proteins were compared with each other by analysing the nitrogen distribution and contents of some amino acids determined colorimetrically. Some marked, but not striking, differences were, however, observed between them.

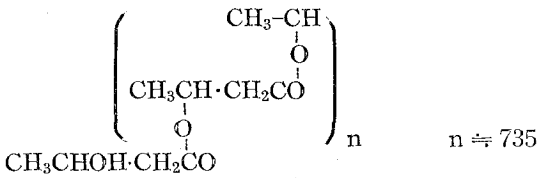
Subtilis Bacteria.

A dry bacterial sample amounting to 530 gs. was made available for our study. The gross percentage composition found is as follows: acetone-soluble fat 2.0,

phospholipid 0.8, wax-like substance (Subtilin, see below.) 3.3, polysaccharide 1.0, nucleoprotein 2.0, free nucleic acid 0.5, protein* 26.3, residue 7.9, ash 4.5.

Fat. ... This substance consisted entirely of glyceride with no accompaniment of sterol. The principal fatty acids were palmitic, lauric, palmitooleic and oleic acids.

Subtilin. ... This is the name designated to the substance which was first found by us as a sole constituent of the wax fraction of subtilis. It also occupies the major part, approximately 60%, of the total lipids of bacteria, and could easily be isolated by extracting microorganism with chloroform after the preliminary elimination of fat and phospholipid with alcohol-ether mixture. In its dry state subtilin is a hard, colorless, plastic-like substance, and slowly dissolves only in chloroform to become viscous fluid from which it is capable to manufacture a very fine fiber or a thin transparent, cellophane-like film. Properties: sp. gr. 1.21, m.p. 200-205°, saponification No. 642, iodine No. 0, optical inactivity, absence of N and P, experimental formula $C_4H_6O_2$, molecular weight 63,000 (viscosimetric method). Upon saponification with 0.5 n alcoholic potash l-β-hydroxybutyric acid and α-crotonic acid were liberated approximately in an equal proportion. From experimental data subtilin might possibly be a polyester of l-β-hydroxybutyric acid, namely $(C_4H_6O_2)_n$, having the theoretical saponification No. 652 and the following structure.



$n \fallingdotseq 735$

Subtilin can be saponified in the following manner:

$$(C_4H_6O_2)n \longrightarrow n/2\ CH_3\cdot CHOHCH_2COOH + n/2\ CH_3CHCH\cdot COOH$$

α-β-hydroxybutyric acid — α-crotonic acid

This interesting substance may be considered the main constituent of the spore membrane of subtilis bacteria, and this seems to be the first example showing the natural occurrence of polyester of any kind.

Nucleoprotein ... Nucleic acid separated from nucleoprotein consisted of 78.7% of ribo- and 10.6% of desoxyribo-nucleic acids. The nucleic acid isolated in a free form was also of a mixed type.

B. Alcaligenes, B. fluorescens, B. Natto, B. Staphylococcus and Sarcina

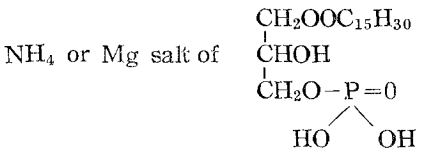
These bacilli were analyzed in the same manner as the research mentioned above. The most conspicuous result was obtained with sarcina phospholipid which was identified as ammonium or magnesium salt of phosphatidic acid. Phosphatidic acid was isolated as lead salt and identified as monophospho-mono-glyceride which was separated upon hydrolysis into α-glycerophosphate, and one unknown fatty acid,

---

* This protein content does not signify the total quantity, since the isolation of protein was carried out by fractional extraction with alkali of varying concentrations and isoelectric precipitation with acid, leaving some protein unprecipitated in solution.

and this new fatty acid was named "sarcinic acid" by the author. This kind of acid is characterized by m.p. 24°, b.p. (methyl ester) 90–105°/2×10^{-3}mm., neutralization No. 233.7 (theoretical value for C_{15}-acid, 232.4), iodine No. 0 and $[\alpha]_D^{27}$ (methyl ester) $=+5.26°$. From these data it may be inferred that sarcinic acid is a sort of pentadecanoic acid of a side chain structure, $C_{15}H_{30}O_2$, and in this connection it may also be considered that occurrence of a lower member of a hypothetical homologous series of odd numbered side chain fatty acids represented by tuberculostearic acid is probable.

The phospholipid of sarcina may be postulated as having the following structural formula;



In the case of staphylococcus phospholipid in addition to sarcinic acid another unidentified fatty acid was isolated.